

Appendix 1

Version With Markings To Show Changes Made in accordance with 37 C.F.R. § 1.121(b)(1)(iii)

In the Specification:

On page 1, line 2, please delete "This is a Continuation-in-Part of co-pending application Serial No. 08/682,853, filed July 12, 1996, which is a Continuation-In-Part of co-pending application Serial No. 08/599,491, filed on January 24, 1996." and insert --The present application is a Continuation of co-pending U.S. Appln. Ser. No. 09/350,309, filed July 9, 1999, which is a Divisional of U.S. Appln. Ser. No. 08/756,386, filed November 29, 1996, now U.S. Patent No. 5,985,557, which is a Continuation-In-Part of U.S. Appln. Ser. No. 08/682,853, filed July 12, 1996, now U.S. Patent No. 6,001,567, which is a Continuation-In-Part of U.S. Appln. Ser. No. 08/599,491, filed January 24, 1996, now U.S. Patent No. 5,846,717.

The invention was made with government support under Cooperative Agreement 70NANB5H1030 awarded by the Department of Commerce, National Institute of Standards and Technology, Advanced Technology Program and Grant No. DE-FG02-94ER81891 awarded by the Department of Energy. The Government has certain rights in the invention.--.

In The Claims:

Please cancel Claims 1-25.

Please add the following claims:

26. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising:

- a) providing:
 - i) a cleavage agent;

- ii) a synthetic target nucleic acid, said synthetic target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;
 - iii) a first oligonucleotide, wherein at least a portion of said first oligonucleotide is completely complementary to said first portion of said first target nucleic acid;
 - iv) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second portion of said target nucleic acid;
- b) mixing said cleavage agent, said synthetic target nucleic acid, said first oligonucleotide and said second oligonucleotide to create a reaction mixture under reaction conditions such that at least said portion of said first oligonucleotide is annealed to said first region of said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage product; and
- c) detecting the cleavage of said cleavage structure.

27. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detecting said non-target cleavage product.

28. The method of Claim 26, wherein said synthetic target nucleic acid comprises an amplified nucleic acid.

29. The method of Claim 28, wherein said amplified nucleic acid is produced using a polymerase chain reaction.

30. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.

31. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

32. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence energy transfer.

33. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge.

34. The method of Claim 26, wherein said first oligonucleotide is attached to a solid support.

35. The method of Claim 26, wherein said second oligonucleotide is attached to a solid support.

36. The method of Claim 26, wherein said cleavage agent comprises a structure-specific nuclease.

37. The method of Claim 36, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

38. The method of Claim 36, wherein said cleavage agent comprises a 5' nuclease.

39. The method of Claim 38, wherein said 5'-nuclease comprises a thermostable 5'-nuclease.

40. The method of Claim 38, wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

41. The method of Claim 40, wherein said thermophilic organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

42. The method of Claim 26, wherein said synthetic target nucleic acid comprises DNA.

43. A kit for detecting the presence of a synthetic target nucleic acid molecule, said synthetic target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region, the kit comprising:

- a) a first oligonucleotide, wherein at least a portion of said first oligonucleotide is completely complementary to said first portion of said first target nucleic acid; and
- b) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second portion of said target nucleic acid.

44. The kit of Claim 43, further comprising a cleavage agent.

45. The kit of Claim 43, wherein said kit further comprises a solid support.

46. The kit of Claim 45, wherein said first oligonucleotide is attached to said solid support.

47. The kit of Claim 43, wherein said second oligonucleotide is attached to said solid support.

48. The kit of Claim 44, wherein said cleavage agent comprises a structure-specific nuclease.

49. The kit of Claim 48, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

50. The kit of Claim 44, wherein said cleavage agent comprises a 5' nuclease.
51. The kit of Claim 50, wherein said 5' nuclease comprises a thermostable 5' nuclease.
52. The kit of Claim 50, wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.
53. The kit of Claim 52, wherein said thermophilic organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.
54. The kit of Claim 49, wherein said structure-specific nuclease comprises a FEN-1 endonuclease.
55. The kit of Claim 43, further comprising a buffer solution.
56. The kit of Claim 55, wherein said buffer solution comprises a source of divalent cations.
57. The kit of Claim 56, wherein said divalent cation is selected from the group consisting of Mn^{2+} and Mg^{2+} ions.
58. The kit of Claim 43, further comprising said target nucleic acid.
59. The kit of Claim 43, further comprising amplification primers.
60. A kit for detecting the presence of a synthetic target nucleic acid molecule comprising a FEN-1 endonuclease and amplification primers.

Appendix 2
Entire Set Of Pending Claims

26. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising:

a) providing:

i) a cleavage agent;

ii) a synthetic target nucleic acid, said synthetic target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;

iii) a first oligonucleotide, wherein at least a portion of said first oligonucleotide is completely complementary to said first portion of said first target nucleic acid;

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second portion of said target nucleic acid;

b) mixing said cleavage agent, said synthetic target nucleic acid, said first oligonucleotide and said second oligonucleotide to create a reaction mixture under reaction conditions such that at least said portion of said first oligonucleotide is annealed to said first region of said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

27. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detecting said non-target cleavage product.

28. The method of Claim 26, wherein said synthetic target nucleic acid comprises an amplified nucleic acid.

29. The method of Claim 28, wherein said amplified nucleic acid is produced using a polymerase chain reaction.

30. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.

31. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

32. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence energy transfer.

33. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, phosphorescence, fluorescence polarization, and charge.

34. The method of Claim 26, wherein said first oligonucleotide is attached to a solid support.

35. The method of Claim 26, wherein said second oligonucleotide is attached to a solid support.

36. The method of Claim 26, wherein said cleavage agent comprises a structure-specific nuclease.

37. The method of Claim 36, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

38. The method of Claim 36, wherein said cleavage agent comprises a 5' nuclease.

39. The method of Claim 38, wherein said 5'-nuclease comprises a thermostable 5'-nuclease.

40. The method of Claim 38, wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

41. The method of Claim 40, wherein said thermophilic organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

42. The method of Claim 26, wherein said synthetic target nucleic acid comprises DNA.

43. A kit for detecting the presence of a synthetic target nucleic acid molecule, said synthetic target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region, the kit comprising:

- a) a first oligonucleotide, wherein at least a portion of said first oligonucleotide is completely complementary to said first portion of said first target nucleic acid; and
- b) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second portion of said target nucleic acid.

44. The kit of Claim 43, further comprising a cleavage agent.

45. The kit of Claim 43, wherein said kit further comprises a solid support.

46. The kit of Claim 45, wherein said first oligonucleotide is attached to said solid support.

47. The kit of Claim 43, wherein said second oligonucleotide is attached to said solid support.

48. The kit of Claim 44, wherein said cleavage agent comprises a structure-specific nuclease.

49. The kit of Claim 48, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

50. The kit of Claim 44, wherein said cleavage agent comprises a 5' nuclease.

51. The kit of Claim 50, wherein said 5' nuclease comprises a thermostable 5' nuclease.

52. The kit of Claim 50, wherein a portion of the amino acid sequence of said nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

53. The kit of Claim 52, wherein said thermophilic organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus*, and *Thermus thermophilus*.

54. The kit of Claim 49, wherein said structure-specific nuclease comprises a FEN-1 endonuclease.

55. The kit of Claim 43, further comprising a buffer solution.

56. The kit of Claim 55, wherein said buffer solution comprises a source of divalent cations.

57. The kit of Claim 56, wherein said divalent cation is selected from the group consisting of Mn²⁺ and Mg²⁺ ions.

58. The kit of Claim 43, further comprising said target nucleic acid.
59. The kit of Claim 43, further comprising amplification primers.
60. A kit for detecting the presence of a synthetic target nucleic acid molecule comprising a FEN-1 endonuclease and amplification primers.

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